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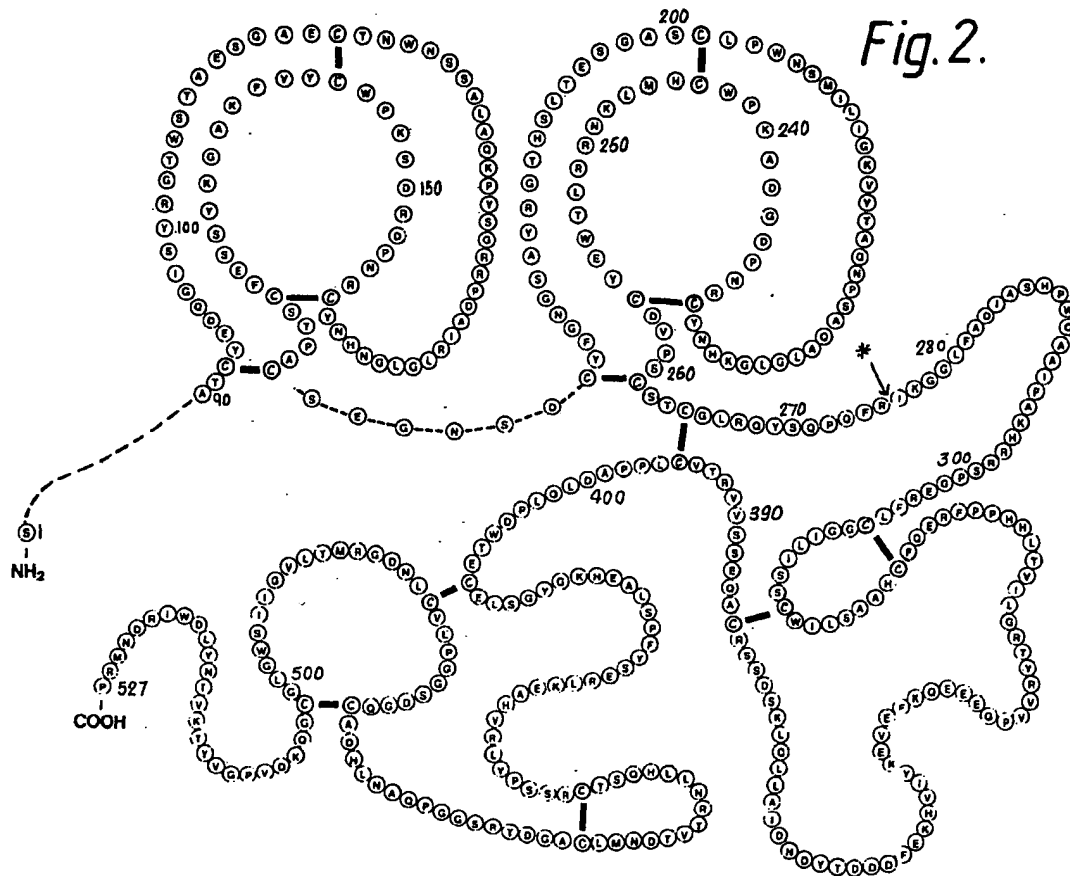
(54) **Medicaments containing tissue plasminogen activator**

(57) Tissue plasminogen activator (t-PA), optionally together with superoxide dismutase (SOD), is used to manufacture a medicament for use in the inhibition of damage to jeopardised tissue during blood reperfusion. The medicament is for preventing damage to tissue following ischaemic attack, e.g. in myocardial tissue, when blood supply is restored.

GB 2 194 886 A

Fig. 1.

Ser Tyr Gln Val Ile Cys Arg Asp Glu Lys Thr Gln Met Ile Tyr Gln Gln His Gln Ser
 1
 Trp Leu Arg Pro Val Leu Arg Ser Asn Arg Val Glu Tyr Cys Trp Cys Asn Ser Gly
 Arg Ala Gln Cys His Ser Val Pro Val Lys Ser Cys Ser Glu Pro Arg Cys Phe Asn
 50
 Gly Gly Thr Cys Gln Gln Ala Leu Tyr Phe Ser Asp Phe Val Cys Gln Cys Pro Glu
 Gly Phe Ala Gly Lys Cys Cys Glu Ile Asp Thr Arg Ala Thr Cys Tyr Glu Asp Gln
 Gly Ile Ser Tyr Arg Gly Thr Trp Ser Thr Ala Glu Ser Gly Ala Glu Cys Thr Asn Trp
 100
 Asn Ser Ser Ala Leu Ala Gln Lys Pro Tyr Ser Gly Arg Arg Pro Asp Ala Ile Arg
 Leu Gly Leu Gly Asn His Asn Tyr Cys Arg Asn Pro Asp Arg Asp Ser Lys Pro Trp
 150
 Cys Tyr Val Phe Lys Ala Gly Lys Tyr Ser Ser Glu Phe Cys Ser Thr Pro Ala Cys
 Ser Glu Gly Asn Ser Asp Cys Tyr Phe Gly Asn Gly Ser Ala Tyr Arg Gly Thr His
 Ser Leu Thr Glu Ser Gly Ala Ser Cys Leu Pro Trp Asn Ser Met Ile Leu Ile Gly Lys
 200
 Val Tyr Thr Ala Gln Asn Pro Ser Ala Gln Ala Leu Gly Leu Gly Lys His Asn Tyr
 Cys Arg Asn Pro Asp Gly Asp Ala Lys Pro Trp Cys His Met Leu Lys Asn Arg Arg
 250
 Leu Thr Trp Glu Tyr Cys Asp Val Pro Ser Cys Ser Thr Cys Gly Leu Arg Gln Tyr
 Ser Gln Pro Gln Phe Arg Ile Lys Gly Gly Leu Phe Ala Asp Ile Ala Ser His Pro Trp
 Gln Ala Ala Ile Phe Ala Lys His Arg Arg Ser Pro Gly Glu Arg Phe Leu Cys Gly
 300
 Gly Ile Leu Ile Ser Ser Cys Trp Ile Leu Ser Ala Ala His Cys Phe Gln Glu Arg Phe
 Pro Pro His His Leu Thr Val Ile Leu Gly Arg Thr Tyr Arg Val Val Pro Gly Glu Glu
 Glu Gln Lys Phe Glu Val Glu Lys Tyr Ile Val His Lys Glu Phe Asp Asp Asp Thr
 350
 Tyr Asp Asn Asp Ile Ala Leu Leu Gln Leu Lys Ser Asp Ser Ser Arg Cys Ala Gln
 Glu Ser Ser Val Val Arg Thr Val Cys Leu Pro Pro Ala Asp Leu Gln Leu Pro Asp
 400
 Trp Thr Glu Cys Glu Leu Ser Gly Tyr Gly Lys His Glu Ala Leu Ser Pro Phe Tyr
 Ser Glu Arg Leu Lys Glu Ala His Val Arg Leu Tyr Pro Ser Ser Arg Cys Thr Ser
 Gln His Leu Leu Asn Arg Thr Val Thr Asp Asn Met Leu Cys Ala Gly Asp Thr Arg
 450
 Ser Gly Gly Pro Gln Ala Asn Leu His Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro
 Leu Val Cys Leu Asn Asp Gly Arg Met Thr Leu Val Gly Ile Ile Ser Trp Gly Leu
 500
 Gly Cys Gly Gln Lys Asp Val Pro Gly Val Tyr Thr Lys Val Thr Asn Tyr Leu Asp
 Trp Ile Arg Asp Asn Met Arg Pro



* Site of cleavage in one-chain t-PA to give two-chain t-PA, in which the A-chain contains the two kringle regions and the B-chain contains the serine protease region.

SPECIFICATION

Novel pharmaceutical use

- 5 The present invention relates to tissue plasminogen activator, to its combination with superoxide 5
dismutase, to pharmaceutical formulations thereof, and to the use thereof in human and veteri-
nary medicine.

There exists a dynamic equilibrium between the enzyme system capable of forming blood
clots, the coagulation system, and the enzyme system capable of dissolving blood clots, the
10 fibrinolytic system, which maintains an intact patent vascular bed. To limit loss of blood from 10
injury, blood clots are formed in the injured vessel. After natural repair of the injury, the
superfluous blood clots are dissolved through operation of the fibrinolytic system. Occasionally,
blood clots form without traumatic injury and may lodge in major blood vessels resulting in a
partial or even total obstruction to blood flow. When this occurs in the heart, lung or brain, the
15 result may be a myocardial infarction, pulmonary embolism or stroke. These conditions combined 15
are the leading cause of morbidity and mortality in the industrialised nations.

Blood clots consist of a fibrous network that is capable of dissolution by the proteolytic
enzyme plasmin. The enzyme is derived from the inactive proenzyme, plasminogen, a component
of blood plasma, by the action of a plasminogen activator. There are two immunologically
20 distinct mammalian plasminogen activators. Intrinsic plasminogen activator, also known as uroki- 20
nase, is an enzyme produced by the kidney and can be isolated from urine. It can also be
prepared from a number of tissue culture sources. Extrinsic plasminogen activator, also known
as vascular plasminogen activator and as tissue plasminogen activator (t-PA), can be isolated
from many tissue homogenates (notably human uterus), the vascular cell wall and from some cell
25 cultures. In addition to these two kinds of plasminogen activator, there is also a bacterial 25
product, streptokinase, prepared from beta-haemolytic streptococci. A major drawback with both
urokinase and streptokinase is that they are active throughout the circulation and not just at the
site of a blood clot. They can, for example, destroy other blood proteins, such as fibrinogen,
prothrombin, factor V and factor VIII so reducing blood clotting ability and increasing the risk of
30 haemorrhage. In contrast, the biological activity of t-PA is dependent on the presence of fibrin to 30
which it binds and where it is activated. Maximum activity is thus developed only at the site of
a blood clot, i.e. in the presence of the fibrin network to be dissolved, and this greatly avoids
the risk of haemorrhage.

The interruption of blood flow in a vessel generally leads to the onset of an ischaemic event.
35 In this condition the tissue is deprived of oxygen and becomes jeopardized, a state in which the 35
tissue is injured but still potentially viable. If however the condition is maintained for a period of,
say, three or more hours, the tissue becomes necrotic and, once in this state, cannot be
recovered. It is therefore important that reperfusion, i.e. the restoration of blood flow, takes
place as soon as possible to salvage the tissue before it becomes permanently damaged.
40 Ironically, reperfusion itself, even if carried out before the tissue becomes necrotic, results in a 40
complex group of phenomena, including the putative formation of the superoxide radical, that
have a deleterious effect on hypoxic tissue. Consequently, reperfusion can lead only to the
partial recovery of jeopardized tissue, the remainder being permanently damaged by the occur-
rence of one or more of these phenomena.

45 It has now been found that t-PA inhibits the damage to jeopardized tissue during reperfusion 45
by protecting it against one or more of the aforementioned phenomena. The mechanism of
action of t-PA in affording such protection has not been elucidated but it is independent of its
action as a thrombolytic agent. This newly discovered property thus enables t-PA, or a pharma-
ceutical formulation thereof, to be used as an inhibitor of damage to jeopardized tissue in the
50 circumstances outlined herein. Accordingly, the present invention provides:— 50

- (a) A method for inhibiting damage to jeopardized tissue during reperfusion in a mammal, which 50
comprises administering to the mammal an effective amount of t-PA;
- (b) Use of t-PA in inhibiting the damage to jeopardized tissue during reperfusion in a mammal;
- (c) Use of t-PA for the manufacture of a medicament for the inhibition of damage to jeopardized 55
tissue during reperfusion in a mammal; or
- (d) A pharmaceutical formulation for use in inhibiting damage to jeopardized tissue during reper- 55
fusion in a mammal, which comprises t-PA and a pharmaceutically acceptable carrier.

Although the present invention may be used to protect any jeopardized tissue, it is particularly
useful in inhibiting damage to jeopardized myocardial tissue.

60 The t-PA of use with the present invention may be any bioactive protein substantially corre- 60
sponding to mammalian, and especially human, t-PA and includes forms with and without
glycosylation. It may be one- or two-chain t-PA, or a mixture thereof, as described in EP-A-112
122, and, in the case of fully glycosylated human t-PA, has an apparent molecular weight on
polyacrylamide gel of about 70,000 and an isoelectric point of between 7.5 and 8.0. Preferably
65 the t-PA has a specific activity of about 500,000 IU/mg (International Units/mg, the International 65

Unit being a unit of activity as defined by WHO, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London, NW3 6RB, U.K.).

The amino acid sequence of t-PA preferably substantially corresponds to that set forth in Fig.

1. The sequence is thus identical to that in Fig. 1 or contains one or more amino acid deletions, 5 substitutions, insertions, inversions or additions of allelic origin or otherwise, the resulting sequence having at least 80%, and preferably 90%, homology with the sequence in Fig. 1 and retaining essentially the same biological and immunological properties of the protein. In particular, the sequence is identical to that in Fig. 1 or has the same sequence but with the amino acid in the 245th position from the serine N-terminus being valine instead of methionine, either sequence optionally having an additional polypeptide N-terminal presequence of Gly-Ala-Arg. 10

The amino acid sequence set forth in Fig. 1 has thirty-five cysteine residues and thus the potential for forming seventeen disulphide bridges. Based on analogy with other proteins whose structure has been determined in more detail, the postulated structure for the sequence (arising from disulphide bond formation) between the amino acid in the 90th position and the proline C-terminus is set forth in Fig. 2. The structure of the N-terminal region is less certain although 15 some proposals have been put forward (*Progress in Fibrinolysis*, 1983, 6, 269-273; and *Proc. Natl. Acad. Sci.*, 1984, 81, 5355-5359). The most important features of the structure of t-PA are the two kringle regions (between the 92nd and the 173rd amino acids and between the 180th and 261st amino acids), which are responsible for the binding of the protein to fibrin, and 20 the serine protease region, which comprises the major part of the B-chain and which is responsible for the activation of plasminogen. The amino acids of special significance in serine proteases are the catalytic triad, His/Asp/Ser. In t-PA these occur at the 322nd, the 371st and the 463rd positions. The disulphide bridge between the 264th and 395th cysteine amino acid residues is also important in that it holds together the A- and the B-chains in the two-chain form 25 of t-PA.

In Figs. 1 and 2, the conventional one and three letter codes have been employed for the amino acid residues as follows:

Asp	D	Aspartic acid	Cys	C	Cysteine	Arg	R	Arginine	
30 Thr	T	Threonine	Val	V	Valine	Lys	K	Lysine	30
Ser	S	Serine	Ile	I	Isoleucine	Trp	W	Tryptophan	
Glu	E	Glutamic acid	Leu	L	Leucine	Gln	Q	Glutamine	
35 Pro	P	Proline	Tyr	Y	Tyrosine	Met	M	Methionine	35
Gly	G	Glycine	Phe	F	Phenylalanine	Asn	N	Asparagine	
Ala	A	Alanine	His	H	Histidine				

40 The t-PA may be obtained by any of the procedures described or known in the art. For example, it may be obtained from a normal or neoplastic cell line of the kind described in *Biochimica et Biophysica Acta*, 1979, 580, 140-153; EP-A-41 766 or EP-A-113 319. It is preferred, however, that t-PA is obtained from a cultured transformed or transfected cell line 45 derived using recombinant DNA technology as described in, for example, EP-A-93 619; EP-A-117 059; EP-A-117 060; EP-A-173 552; EP-A-174 835; EP-A-178 105; WO 86/01538; WO 86/05514; or WO 86/05807. It is particularly preferred that Chinese hamster ovary (CHO) cells are used for the production of t-PA and are derived in the manner as described in *Molecular and Cellular Biology*, 1985, 5(7), 1750-1759. In this way, the cloned gene is cotransfected with the 50 gene encoding dihydrofolate reductase (dhfr) into dhfr⁻ CHO cells. Transformants expressing dhfr are selected on media lacking nucleosides and are exposed to increasing concentrations of methotrexate. The dhfr and t-PA genes are thus coamplified leading to a stable cell line capable of expressing high levels of t-PA.

The t-PA is, preferably, purified using any of the procedures described or known in the art, 55 such as the procedures described in *Biochimica et Biophysica Acta*, 1979, 580, 140-153; *J. Biol. Chem.*, 1979, 254(6), 1998-2003; *ibid*, 1981, 256(13), 7035-7041; *Eur. J. Biochem.*, 1983, 132, 681-686; EP-A-41 766; EP-A-113 319; or GB-A-2 122 219.

t-PA may be used in the manner of the present invention either alone or in combination with another therapeutic agent which also inhibits damage to jeopardized tissue during reperfusion. A particularly useful example of such a combination is with superoxide dismutase (SOD), an enzyme 60 that is known to scavenge and destroy superoxide radicals, one of the phenomena capable of causing tissue damage. Indeed, it has also been found that the combination of t-PA and SOD provides a significantly potentiated level of inhibition compared with that provided by t-PA or SOD *per se*. Accordingly the present invention also provides a combination of t-PA and SOD.

65 The combination of t-PA and SOD affords a particularly convenient means both for the 65

removal of blood clots and for the inhibition of damage to jeopardized tissue during subsequent reperfusion. Thus, the administration of t-PA and SOD will result first in the removal of the blood clot through the known thrombolytic action of t-PA and then in the inhibition of tissue damage through the combined action of t-PA and SOD.

- 5 The SOD of use in combination with t-PA may be any bioactive protein substantially corre-
sponding to any one or more of a group of enzymes known generally by this name. It is
preferably of mammalian, and especially of bovine or human, origin and is generally associated
with a metal cation by which it is normally classified. Examples of a metal cation include iron,
manganese, copper and preferably combinations of copper with other metals, such as zinc,
10 cadmium, cobalt or mercury, of which a copper/zinc combination is preferred. Both the mangan-
ese and the copper/zinc forms of SOD occur naturally in humans. The iron and manganese
forms of SOD of bacterial origin both have a molecular weight of about 40,000 and are dimers.
The manganese form of SOD of eukaryotic origin on the other hand has a molecular weight of
about 80,000 and is a tetramer. The copper/zinc form of SOD of eukaryotic origin has a
15 molecular weight of about 32,000 and is a dimer with one copper cation and one zinc cation
per subunit. The copper cation is ligated to four histidine residues per subunit and the zinc
cation is ligated between histidine and aspartic acid. There is also a copper/zinc form of SOD of
eukaryotic origin which has a molecular weight of about 130,000 and which consists of four
subunits. The molecular weights of the various forms of SOD were estimated using sedimenta-
20 tion equilibrium, molecular sieving or using polyacrylamide gels. The isoelectric points of the
various forms of SOD range from 4 to 6.5 depending on the degree of sulphation and/or
deamidation. Preferably, the specific activity of the copper/zinc form of SOD of bovine or human
origin is at least 3000 U/mg (the unit of activity being as defined in *J.Biol. Chem.*, 1969, 244,
6049-6055).
- 25 The amino acid sequence of the copper/zinc form of SOD of bovine or human origin preferably
substantially corresponds to that set forth in *J. Biol. Chem.*, 1974 249(22), 7326 to 7338, in
the case of that of bovine origin, and *Biochemistry*, 1980, 19, 2310 to 2316 and *FEBS Letters*,
1980, 120, 53 to 55, in the case of that of human origin. The sequence is thus identical to that
set forth in these articles or contains one or more amino acid deletions, substitutions, insertions,
30 inversions or additions of allelic origin or otherwise, the resulting sequence having sufficient
homology with the published sequence so as to retain essentially the same biological and
immunological properties.

The amino acid sequence of the copper/zinc form of SOD of bovine origin contains three
cysteine residues per subunit (*J. Biol. Chem.*, 1974, 249(22), 7326-7338). The intrachain
35 disulphide bridge occurs between the Cys 55 and Cys 144 residues while the interchain disul-
phide bridge occurs between the Cys 6 residues. The amino acid sequence of the copper/zinc
form of SOD of human origin contains four cysteine residues per subunit (*Biochemistry*, 1980,
19, 2310 to 2316 and *FEBS Letters*, 1980, 120, 53 to 55). The intrachain disulphide bridge
occurs between the Cys 57 and Cys 146 residues while the interchain disulphide bridge occurs
40 also between the Cys 6 residues. The Cys 111 residue remains free.

The SOD may be obtained by any suitable procedure described or known in the art. For
example, it may be obtained from erythrocytes or from liver by an extraction procedure of the
kind described in GB-A-1 407 807 and GB-A-1 529 890. Alternatively, SOD may be obtained
from a cultured transformed or transfected cell line, derived using recombinant DNA technology
45 as described in, for example, Australian patent application 27461/84, WO 85/01503, EP-A-138
111, EP-A-164 566, EP-A-173 280 and EP-A-180 964.

The SOD is preferably purified using any suitable procedure described or known in the art,
such as the procedure described in EP-A-112 299.

In using t-PA, or a combination of t-PA and SOD, in the manner of the present invention, it is
50 preferred to employ the active ingredient(s) in the form of a pharmaceutical formulation. In the
case of the combination, the active ingredients may be employed in separate formulations which
may be administered simultaneously or sequentially. If they are administered sequentially, it is
preferred to administer the t-PA formulation first and then the SOD formulation. In any event, the
delay in administering the second of the two formulations should not be such as to lose the
55 benefit of a potentiated effect of the combination of the active ingredients *in vivo* in inhibiting
tissue damage. However, rather than use separate formulations, it is much more convenient to
present both active ingredients together in a single combined formulation. Accordingly, the
present invention also provides a pharmaceutical formulation, which comprises t-PA and SOD
and a pharmaceutically acceptable carrier.

60 Generally, t-PA, or the combination of t-PA and SOD, will be administered by the intravascular
route, whether by infusion or by bolus injection, and thus a parenteral formulation is required. It
is preferred to present a lyophilised formulation to the physician or veterinarian because of the
significant transportation and storage advantages that it affords. The physician or veterinarian
may then reconstitute the lyophilised formation in an appropriate amount of solvent as and when
65 required.

Parenteral and lyophilised pharmaceutical formulations containing t-PA are known in the art. Examples of such art include EP-A-41 766; EP-A-93 619; EP-A-112 122; EP-A-113 319; EP-A-123 304; EP-A-143 081; EP-A-156 169; WO 86/01104; Japanese patent publication 57-120523 (application 56-6936) and Japanese patent publication 58-65218 (application 56-163145). Additional preferred examples include GB-A-2 176 702 and GB-A-2 176 703. All such formulations are also suitable for SOD and for the combination of t-PA and SOD.

Intravascular infusions are normally carried out with the parenteral solution contained within an infusion bag or bottle or within an electrically operated infusion syringe. The solution may be delivered from the infusion bag or bottle to the patient by gravity feed or by the use of an infusion pump. The use of gravity feed infusion systems does not afford sufficient control over the rate of administration of the parenteral solution and, therefore, the use of an infusion pump is preferred especially with solutions containing relatively high concentrations of active ingredients. More preferred, however, is the use of an electrically operated infusion syringe which offers even greater control over the rate of administration.

An effective amount of t-PA, and of a combination of t-PA and SOD, to inhibit damage to jeopardized tissue during reperfusion will of course depend upon a number of factors including, for example, the age and weight of the mammal, the precise condition requiring treatment and its severity, the route of administration, and will ultimately be at the discretion of the attendant physician or veterinarian. An effective dose, however, in the case of t-PA is generally in the range from 0.2 to 4 mg/kg (i.e. 100,000 to 2,000,000 IU/kg assuming a specific activity for t-PA of 500,000 IU/mg), preferably from 0.3 to 2 mg/kg (i.e. 150,000 to 1,000,000 IU/kg), bodyweight of patient per hour. Thus for a 70kg adult human being, an effective amount per hour will preferably be from 20 to 140 mg (i.e. 10,000,000 to 70,000,000 IU), especially about 70 mg (i.e. 35,000,000). If SOD is used in combination with t-PA, then an effective dose of SOD is generally in the range of from 1000 to 50,000 U/kg, preferably from 7000 to 20,000 U/kg, bodyweight of patient per hour. Thus for a 70 kg adult human being, an effective amount of SOD per hour will preferably be from 500,000 to 1,500,000 U.

The following examples are provided in illustration of the present invention and should not be construed in any way as constituting a limitation thereof.

Example 1: Preparation of Parenteral Formulation of t-PA

A parenteral formulation of t-PA was prepared substantially as described in GB-A-2 176 703. The t-PA had a specific activity of about 300,000 IU/mg.

Example 2: Preparation of Parenteral Formulation of SOD

Bovine SOD of the copper/zinc form was obtained from Sigma Chemical Co., St Louis, Missouri, U.S.A., 63178, as a powder and dissolved in substantially neutral physiological saline solution.

Example 3: Preparation of Parenteral Formulation of t-PA and SOD

The formulations of Examples 1 and 2 were mixed and further diluted in physiological saline solution to achieve the required dosage.

Example 4: Protection of Jeopardized Tissue by t-PA and by t-PA and SOD

(a) Methodology

Male beagle dogs (10-12kg) were anaesthetized with pentobarbital sodium, intubated, and ventilated with room air via a Harvard respirator. Catheters for infusion and arterial blood pressure measurements were implanted in the left jugular vein and left carotid artery. A thoracotomy was performed at the fourth intercostal space, the heart suspended in a pericardial cradle and the left anterior descending artery (LAD) isolated just below the first major diagonal branch. An electromagnetic flow probe was placed on the LAD. A 90 minute occlusion of the LAD was produced by placing a snare of I/O silk suture distal to the flow probe. Treatment was initiated intravenously fifteen minutes prior to release of this snare occlusion and continued for 45 minutes after release. The thoracotomy was closed, and the animals were allowed to recover from the surgical procedures. The animals were reanaesthetized 24 hours after the occlusion, and the flow in the LAD reassessed. Then the heart was removed for post mortem quantification of infarct size.

Four groups of dogs were evaluated. Group 1 consisted of saline controls. Group II were administered 2.5 mg/kg (750,000 IU/kg) t-PA, Group III were administered 16,500 U/kg bovine SOD, and Group IV were administered both 2.5 mg/kg (750,000 IU/kg) t-PA and 16,500 U/kg bovine SOD. The formulations used were as described in Examples 1 to 3.

Myocardial infarct size was quantified by an ex vivo dual reperfusion technique. Cannulas were inserted into the LAD immediately distal to the site of occlusion and into the aorta above the coronary ostia. The LAD coronary bed that was perfused with 1.5% triphenyl tetrazolium

hydrochloride (TTC) in 0.02 M potassium phosphate buffer, pH 7.4. The aorta was perfused in a

retrograde manner with 0.5% Evans blue dye. Both regions were perfused with their respective stains at a constant pressure of 100 mm mercury for five minutes. The heart was cut into 8 mm slices perpendicular to the apex-base axis. The area of the left ventricle at risk of infarction due to anatomical dependence on the LAD for blood flow is identified by the lack of Evans blue

5 in this region. The region of infarcted myocardium within the area at risk was demarcated by the lack of staining of the tissue when perfused with TTC due to a loss of dehydrogenase enzymes. 5

The transverse ventricular sections were traced carefully on to clear acrylic overlays to provide a permanent record of infarct morphology and to allow planimetric confirmation of infarct size. Ventricular sections then were trimmed of right ventricular muscle, valvular, and fatty tissue. The 10 total left ventricle area at risk and infarct was separated by careful dissection and weighed. 10

Infarct size was expressed as a percentage of the anatomic area at risk. Statistical comparison of the drug treatment group to the control group was made using a one way analysis of variance (anova) using Bonferroni's method for multiple comparison (*Circulation Research*, 1980, 47, 1-9). A p value of less than 0.05 was taken as the criterion of significance. 15

15 (b) Results

TABLE

20					20				
-----					-----				
GROUP		NUMBER	AREA AT RISK	% VENTRICLE*					
		OF DOGS	INFARCTED	AT RISK					
25					25				
-----					-----				
I.	Saline	5	36.0 ± 8.9	37.3 ± 7.6					
30									
II.	t-PA	6	14.3 ± 11.7	35.7 ± 5.4					
35									
III.	SOD	4	13.0 ± 4.6	30.6 ± 2.6					
40									
IV.	t-PA+	3	2.3 ± 1.3	37.2 ± 9.1					
	SOD								
40					40				
-----					-----				

45 * Data are expressed as means ± standard errors. 45

The proportion of the left ventricle made ischaemic by mechanical occlusion of the LAD was 50 not significantly different between any of the treatment groups and the control group by ANOVA. 50

(c) Conclusions

The use of t-PA significantly inhibited the myocardial infarct size thus demonstrating its ability 55 to protect jeopardized tissue during reperfusion. In addition, the combined use of t-PA and SOD achieved a synergistic inhibitory effect in this regard, with the combination providing a level of inhibition greater than that provided by each of t-PA or SOD on its own. 55

CLAIMS

- 60 1. Use of t-PA for the manufacture of a medicament for the inhibition of damage to jeopardized tissue during reperfusion in a mammal. 60
2. Use of t-PA and SOD for the manufacture of a medicament for the inhibition of damage to jeopardized tissue during reperfusion in a mammal.
3. Use of t-PA and SOD for the manufacture of a medicament for the removal of a blood 65 clot and for the inhibition of damage to jeopardized tissue during reperfusion in a mammal. 65

4. Use according to any one of the preceding claims, wherein the tissue is myocardial tissue.
5. Use according to any one of the preceding claims, wherein the t-PA is in the one-chain form.
6. Use according to any one of claims 1 to 4, wherein the t-PA is in the two-chain form.
- 5 7. Use according to claim 5 or claim 6, wherein the t-PA has the amino acid sequence set forth in Fig. 1 or has the same amino acid sequence but with the amino acid in the 245th position from the serine N-terminus being valine instead of methionine, either sequence optionally having an additional polypeptide N-terminal presequence of Gly-Ala-Arg. 5
8. Use according to claim 2 or claim 3, wherein the SOD is the copper/zinc form of bovine or human origin. 10
9. A combination of t-PA and SOD.
10. A combination of t-PA and SOD for use in human and veterinary medicine.
11. A combination of t-PA and SOD for use in the inhibition of damage to jeopardized tissue during reperfusion in a mammal.
- 15 12. A combination of t-PA and SOD for use in the removal of a blood clot and in the inhibition of damage to jeopardized tissue during reperfusion in a mammal. 15
13. A combination according to any one of claims 9 to 12, wherein the t-PA is in the one-chain form.
14. A combination according to any one of claims 9 to 12, wherein the t-PA is in the two-chain form. 20
15. A combination according to claim 13 or claim 14, wherein the t-PA has the amino acid sequence set forth in Fig. 1 or has the same amino acid sequence but with the amino acid in the 245th position from the serine N-terminus being valine instead of methionine, either sequence optionally having an additional polypeptide N-terminal presequence of Gly-Ala-Arg.
- 25 16. A combination according to any one of claims 9 to 12, wherein the SOD is the copper/zinc form of bovine or human origin. 25
17. A pharmaceutical formulation, which comprises a combination according to any one of claims 9 to 16 and a pharmaceutically acceptable carrier.